# MOLECULAR CONFIRMATION OF UNIDIRECTIONAL HYBRIDIZATION IN *BEGONIA* × *TAIPEIENSIS* PENG (BEGONIACEAE) FROM TAIWAN[1]

*Ching-I Peng[2] and Tzen-Yuh Chiang[3]*

## ABSTRACT

An unusual *Begonia* that sheds staminate flowers prematurely at bud stage was collected from several localities in northern Taiwan. Observations on morphology, pollen stainability, and seed set of this species initially suggested a hybrid origin. Morphological comparisons, distribution patterns, chromosome cytology, and experimental hybridization showed that such plants are $F_1$ hybrids ($2n = 41$) between *Begonia formosana* (Hayata) Masamune ($n = 30$) and *B. aptera* Blume ($n = 11$), both of which are widespread in Taiwan and sympatric in most of their ranges. These hybrids were named *Begonia* × *taipeiensis* Peng. Experimental crosses between the putative parental species consistently resulted in germinable seeds and healthy $F_1$ plants only when *B. formosana* was used as the female parent. Molecular data obtained from sequences of the *atp*B-*rbc*L spacer of chloroplast DNA confirmed that unidirectional hybridization between the putative parents in the wild resulted in the formation of *B.* × *taipeiensis*. No natural hybrid populations with a maternal origin from *B. aptera* have been detected. Abortion caused by a post-pollination barrier occurs when *B. aptera* was used as a maternal parent. Low pollen fertility of $F_1$ hybrids indicates that the natural hybrid is maintained by recurrent hybridization between the parental species.

*Key words*: *atp*B-*rbc*L noncoding spacer, *Begonia* × *taipeiensis*, *B. formosana*, *B. aptera*, chloroplast DNA, cytology, directional hybridization, natural hybrid, Taiwan.

Natural hybridization, one of the most influential processes that increases species diversity and stabilizes genetic complexity via genetic recombination or introgression (Arnold, 1992, 1993), occurs frequently in plants (P. S. Soltis & D. E. Soltis, 1991; P. S. Soltis et al., 1992; Arnold, 1997). Studies on the origins of natural hybrids and their genetic composition frequently raise fundamental questions concerning reproductive barriers, survivorship and fitness of the hybrids, and mechanisms of reticulate evolution. In many cases (e.g., Louisiana Irises; Arnold & Bennett, 1993) natural selection may favor hybrid plants of specific maternal origin. It is essential to study the genetic makeup of natural hybrids in order to elucidate evolutionary processes and the ecological adaptation of these plants.

It has been abundantly demonstrated that natural hybridization plays a major role in the evolution of species groups or complexes (*Ludwigia*: Peng, 1988, 1990; *Iris*: Arnold et al., 1990; *Glycine*: Doyle et al., 1990; *Helianthus*: Rieseberg et al., 1990, Rieseberg, 1991; *Gossypium*: Wendel et al., 1991; *Senecio*: Harris & Ingram, 1992; *Allium*: Ohsumi et al., 1993; *Leucaena*: Hughes & Harris, 1994; *Arabidopsis*: Mummenhoff & Hurka, 1995; *Argyroxiphium*: Baldwin, 1997; and *Argyranthemum*: Francisco-Ortega et al., 1997). Natural hybridization occurs frequently in Taiwanese *Begonia* (Y. K. Chen, 1988; Peng & Chen, 1991; Peng & Sue, 2000), which may have resulted in the high level of endemism of this genus on the island (66.7%; cf. C. H. Chen, 1993), while causing difficulties in clarifying phylogenetic relationships (see Rieseberg & Morefield, 1995).

*Begonia* × *taipeiensis* Peng was recently described as a new intersectional hybrid (Peng & Sue, 2000). Geographical distribution, low level of pollen stainability, and intermediate morphological characters between *B. formosana* (Hayata) Masamune and *B. aptera* Blume initially suggested such an origin. *Begonia aptera* [sect. *Sphenanthera* (Hassk.) Warb.] is distributed in northern Taiwan,

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[2] Institute of Botany, Academia Sinica, Nankang, Taipei 115, Taiwan.

[3] Department of Biology, National Cheng-Kung University, Tainan 700, Taiwan.

while *B. formosana* [sect. *Platycentrum* (Klotzsch) A. DC.] is widespread in northern and eastern Taiwan. Both species occur in wide altitudinal ranges (50–2000 m). They frequently co-occur throughout their range of overlap (Fig. 1). The flowering periods are March to December for *B. formosana*, June to August for *B. aptera*, and April to December for *B.* × *taipeiensis*. Two discrete populations of *Begonia* × *taipeiensis* were found mixed with its putative parents in the Taipei Basin of northern Taiwan. *Begonia formosana* is characterized by having creeping rhizomes with ascending to erect flowering stems, broadly ovate, lobed leaves, 2-locular ovaries, and unequally winged capsules, the abaxial one being very pronounced. In contrast, *B. aptera* is a tall, cane species lacking horizontal rhizomes, has lanceolate leaves, 3-locular ovaries, and rounded, wingless capsules. *Begonia* × *taipeiensis* has short rhizomes, intermediate plant height and leaf shape, and 2-locular ovaries (these occasionally highly reduced and without locules) with diminutive wings. Like all other species of *Begonia*, *B.* × *taipeiensis* is monoecious and interflorally protandrous. At anthesis, it produces abundant flowers of both sexes. All staminate flowers observed develop normally up to their late bud stages and then drop off before they open. All *Begonia* of Taiwan are frequently pollinated by blowflies (Calliphoridae), hover flies (Syrphidae), and honeybees (Apidae) (Y. K. Chen, 1988). Plants of *Begonia* often grow along moist stream banks. Their light seeds float and are carried easily by water currents. Seeds of *Begonia* are primarily dispersed with the aid of wind (van der Pijl, 1972).

Traditionally, morphological examination and chromosome cytology have provided useful information in revealing putative parents. Nevertheless, such data are not sufficient to determine the direction of hybridization and to detect genetic variability. Recently, many convincing studies using molecular techniques such as comparison of RFLP patterns of ribosomal DNA (Arnold et al., 1990; Stein & Barrington, 1990; Rieseberg, 1991; P. S. Soltis & D. E. Soltis, 1991; Hughes & Harris, 1994; Mummenhoff & Hurka, 1995) and those of organelle DNA (D. E. Soltis & P. S. Soltis, 1989; DeMarais et al., 1992; Garcia & Davis, 1994; Wang & Szmidt, 1994; Brochmann et al., 1998), coupled with experimental hybridizations (Hodges et al., 1996), have been conducted to address reticulate or polyploid evolution (D. E. Soltis & P. S. Soltis, 1993). Chloroplast and mitochondrial genomes, being maternally inherited in most organisms (Chiu & Sears, 1985; D. E. Soltis et al., 1990), have been useful in elucidating the direction of gene flow between populations (Whittemore & Schaal, 1991) and in detecting the maternal origin of natural hybrids (Wendel et al., 1991). Thus, in this study, a noncoding spacer region between the genes *rbc*L and *atp*B of the chloroplast genome, which has been used as a molecular marker at the species level (Golenberg et al., 1993; Chiang, 1994; Manen & Natali, 1995; Chiang et al., 1998), was sequenced.

In this study the following questions will be addressed: (1) Did *B.* × *taipeiensis* originate from interspecific hybridization between *B. aptera* and *B. formosana*? (2) Do hybrids with alternate maternal origins have the same survivorship? (3) Do hybrids backcross with putative parents and produce viable offsprings? (4) Is the frequency of occurrence of natural hybrids a reflection of the ease of experimental crosses?

## Materials and Methods

### 1. Materials sampled

Major herbaria in Taiwan (HAST, NTUF, TAI, TAIF, TNM) were consulted to determine the distribution and phenology of *Begonia* × *taipeiensis* and its putative parents, *B. formosana* and *B. aptera*. Living plants of the hybrid and its associated putative parents were collected from two localities of Taipei County, Hsichih and Wulai (Fig. 1). Additionally, a plant each of *B. formosana* and *B. aptera* from Chiufen and Nankang, respectively, where *B.* × *taipeiensis* was not found, were collected for comparison (Fig. 1). All plants were grown in the experimental greenhouse of the Institute of Botany, Academia Sinica, Taipei, for cytological and molecular examination and for experimental hybridization. *Begonia palmata* D. Don, a congener collected from Hsitou, Nantou County, was used as an outgroup. Herbarium vouchers were deposited at HAST (Table 1).

### 2. Experimental pollination

Experimental self-pollinations of *B. aptera* and *B. formosana* were done. Reciprocal crosses were carried out between *B. formosana* and *B. aptera* from the same locality and from different localities in the greenhouse. *Begonia* × *taipeiensis* collected from the wild was used as a pistillate parent for backcrossing with both putative parents. The fact that *Begonia* × *taipeiensis* shed staminate flowers prematurely precludes the possibility of using it as a pollen donor. Seeds ($F_1$ progeny) were grown to maturity to examine the fertility of pollen grains and for cytological study.

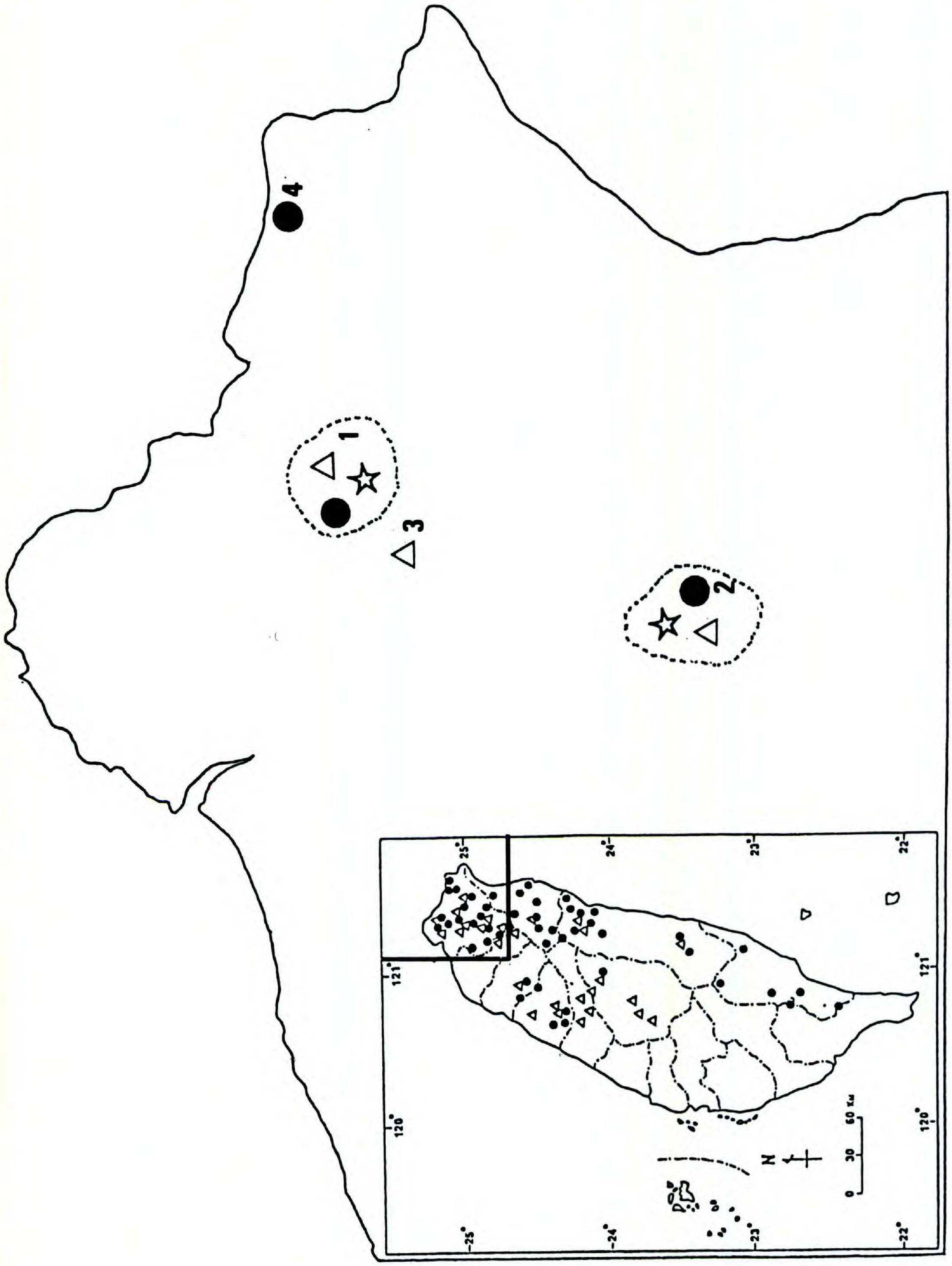


Figure 1. Map showing the distribution (inset) of *Begonia formosana* (circles) and *B. aptera* (triangles) in Taiwan; and location of the specimens collected from localities (1–4) for cytological and molecular studies: *B. formosana* (circles), *B. aptera* (triangles), and *B.* × *taipeiensis* (stars); 1, Wulai; 2, Hsichih; 3, Nankang; 4, Chiufen.

Table 1. Materials of *Begonia* × *taipeiensis* and its putative parents for cytological and molecular analysis. Herbarium vouchers are deposited at HAST.

| Taxa | Localities | Vouchers | EMBL accession no. |
|---|---|---|---|
| *B.* × *taipeiensis* | 1. Wulai, Taipei Co. | *Peng 13899* | AJ009601 |
| | | *Peng 15106* | AJ009602 |
| | 2. Hsichih, Taipei Co. | *Peng 16320* | AJ009600 |
| *B. aptera* | 1. Wulai, Taipei Co. | *Peng 16276* | AJ223092 |
| | | *Peng 16153* | AJ009606 |
| | 2. Hsichih, Taipei Co. | *Peng 16321* | AJ009605 |
| | 3. Nankang, Taipei Co. | *Peng 16292* | AJ009604 |
| *B. formosana* | 1. Wulai, Taipei Co. | *Peng 13915* | AJ009599 |
| | 2. Hsichih, Taipei Co. | *Peng 16319* | AJ009598 |
| | 3. Chiufen, Taipei Co. | *Leu 867* | AJ009597 |
| *B. palmata* | 1. Chitou, Nantou Co. | *Peng 16081* | AJ007745 |
| | 2. Tengchi, Kaohsiung Co. | *Peng 16831* | AJ242856 |
| Experimental hybrid: | | | |
| *Bf*×*a*-1: *B. formosana* × *B. aptera* = *Leu 867* × *Peng 16153* | | | AJ242857 |
| *Bf*×*a*-2: *B. formosana* × *B. aptera* = *Peng 13915* × *Peng 16153* | | | AJ242858 |

### 3. CHROMOSOME CYTOLOGY

Flower buds to be examined for meiotic behavior were fixed in a 3 : 1 mixture of 95% ethanol and glacial acetic acid and stored in the refrigerator. Prior to staining, the buds were hydrolyzed for 5–8 min. at 60°C using a 1 : 1 mixture of concentrated HCl and 95% ethanol. They were then squashed in FLP orcein (Jackson, 1973). Somatic chromosome counts were obtained from actively growing root tips pretreated for 3 to 4 hr. in 8-hydroxyquinoline, then fixed as above for at least 10 min. The root tips were then hydrolyzed in 1 N HCl for 8–10 min. at 60°C and squashed in FLP orcein. All analyzable chromosome configurations (mostly diakinesis or metaphase I) were documented with camera lucida drawings or photomicrographs using Kodak Panatomic-X films. Negatives and drawings are deposited at the Institute of Botany, Academia Sinica, Taipei.

### 4. MOLECULAR METHODS

*DNA isolation, PCR, and nucleotide sequencing.* Fresh tissue of young shoots was ground in liquid nitrogen and stored at −70°C for DNA extraction. Genomic DNA was extracted following a CTAB methodology (Doyle & Doyle, 1989). PCR was performed in a volume of 100 μl reaction using 10 ng of template DNA, 10 μl of 10× buffer, 10 μl $MgCl_2$ (25 mM), 10 μl dNTP mix (8 mM), 10 pmole of each primer, 10 μl of 10% NP-40, and 2 U of *Taq* polymerase (Promega, Madison, U.S.A.; cf. Chien et al., 1976). The reaction was programmed on an MJ Thermal Cycler (PTC 100) as one cycle of denaturation at 95°C for 4 min., 30 cycles of denaturation at 92°C for 45 sec., annealing at 52°C for 1 min. 15 sec., and extension at 72°C for 1 min. 30 sec., followed by a 10 min. extension. Template DNA was denatured with reaction buffer, $MgCl_2$, NP-40, and ddH2O for 4 min. (first cycle), and cooled on ice immediately. Primers [*rbc*L-1, 5′-AACACCAGCTTTA(G)AATCCAA-3′; *atp*B-1, ACATCT(G)AA(G)TACT(G)GGACCAATAA-3′; (Chiang et al., 1998)], dNTPs, and *Taq* polymerase were added to the above ice-cold mix. Reaction was restarted at the first annealing at 52°C. PCR fragments were eluted using High Pure PCR Product Purification Kit (Boehringer Mannheim, Germany; cf. Vogelstein & Gillespie, 1979). PCR products were ligated to a pT7blue T-vector (Novagen, Madison, U.S.A.; cf. Marchuk et al., 1991) and cloned in competent *E. coli* DH5α. Plasmid DNA was extracted from transformed cells and sequenced with $^{32}P$ labeling extension/termination reaction (Sanger et al., 1977) using *fmol* DNA Sequencing System (Promega, Madison, U.S.A.; cf. Murray, 1989). For completing sequencing, with about 75 base pair overlaps from both ends, two additional primers, i.e., *rbc*L-*2* (5′-GGTTCGTTCTTTGAACATGG-3′) and *atp*B-*2* (5′-CCGTCCAACCGAATCCAATTC-3′), were synthesized.

*DNA alignment and phylogenetic analysis.* Alignment of nucleotide sequences was performed by Clustal V program (Higgins et al., 1992) and improved by eye. Parsimony phylogenetic anal-

yses were performed by using the Phylogenetic Analysis Using Parsimony Program (PAUP, version 3.1.1., Swofford, 1993) with the branch and bound algorithm. Neighbor-Joining (NJ) analysis by calculating Kimura's (1980) two-parameter distance was also performed using Molecular Evolutionary Genetics Analysis Program (MEGA, version 1.01, Kumar et al., 1993). Confidence of clades reconstructed was tested by bootstrapping (Felsenstein, 1985) with 1000 replicates (Hedges, 1992). The nodes with bootstrap values greater than 0.70 are significantly supported with > 95% probability (Hillis & Bull, 1993). In NJ analysis, complete-and-partial (CP) bootstrap technique was used to correct the bias (conservativeness) of the standard bootstrap approach (Li & Zharkikh, 1995). A g1 test (Huelsenbeck, 1991) of skewed tree-length distribution was calculated from 10,000 random trees generated by PAUP in order to measure the information content of the data. Critical values of the g1 test were obtained from Hillis and Huelsenbeck (1992). The fit of character data on phylogenetic hypotheses (Swofford, 1991) was evaluated by the consistency index, CI (Kluge & Farris, 1969), and the retention index, RI (Farris, 1989). The statistical significance of the CI was determined according to the method of Klassen et al. (1991). The number of nucleotide substitution, which is the number of transitional and transversional substitutions per site, was calculated following the methodology of Wu and Li (1985).

## Results

### EXPERIMENTAL SELFING AND HYBRIDIZATION

In all experimental geitonogamous selfing attempts made on *Begonia formosana* and *B. aptera*, fruits with 95–100% viable seeds were consistently obtained. Plants of *B. formosana* required 30–45 days for fruit maturation, whereas those of *B. aptera* required 90–150 days. *Begonia formosana* and *B. aptera* were crossed reciprocally in the experimental greenhouse. Nearly all crosses using *B. formosana* as pistillate parent were successful, producing mature fruits with 80–90% plump seeds 35–45 days after pollination. Such seeds were viable and flowering artificial $F_1$ hybrids were readily obtained from them. The level of stainable pollen in these $F_1$ plants was extremely low (ranging from 0 to 5%), which agrees with that of the naturally occurring *B.* × *taipeiensis*. When *B. aptera* was used as the pistillate parent in the crosses, however, precocious fruit drop occurred ca. 60 days after artificial pollination.

Experimental hybrids between *B. formosana* and *B. aptera* were grown to maturity. They flowered annually and have persisted in the mist greenhouse since the summer of 1995. The plant height in these artificial hybrids was within the variation range of naturally occurring *B.* × *taipeiensis*. The artificial hybrids closely resembled *B.* × *taipeiensis* in habit and details of vegetative as well as floral characters. When plants of *Begonia* × *taipeiensis* collected from the wild were backcrossed (as pistillate parent) to *B. formosana* and *B. aptera*, respectively, fruit set was successfully obtained. However, five backcrossing attempts made with *B. formosana* and 11 such attempts with *B. aptera* produced fruits with zero or negligible plump seeds that failed to germinate.

### CHROMOSOME CYTOLOGY

Previous studies revealed a meiotic chromosome number of $n = 11$ in *B. aptera*, $n = 30$ in *B. formosana*, and $2n = 41$ in *B.* × *taipeiensis* (Peng & Sue, 2000). Like *B.* × *taipeiensis*, experimental hybrids of *B. formosana* × *B. aptera* consistently have a somatic chromosome number of $2n = 41$. Also, abnormalities in chromosome configurations were observed. Meiotic chromosome configurations of both *B.* × *taipeiensis* and *B. formosana* × *B. aptera* typically consisted of some sticky, often disoriented bivalents, univalents, and multivalent associations (Fig. 2).

### MOLECULAR DATA

Sequences with a consensus length of 854 base pairs of the *atp*B-*rbc*L spacer were obtained from *B.* × *taipeiensis* and its putative parents. Fifteen variable sites were found between sequences (Table 2). That this chloroplast spacer has, on average, 34.0% A and 36.1% T agrees with one of the common properties, i.e., AT-rich, of most noncoding spacers (Li, 1997). Differences in the rate of nucleotide substitution (Table 3) among species of *Begonia* we sampled ranged from 0.0017 (between *B. aptera* and *B. formosana*) to 0.0056 (between *B. aptera* and *B. palmata*) (mean = 0.0033).

Of the three individuals of *B. formosana* we examined, two had identical nucleotide sequences (Tables 1, 2). The third collection from Chiufen (*Leu 867*) differed at two different positions (bp 191 and 762). Interpopulational variation was present in *B. aptera* at bp 275 and 794. In our study, sequences of this chloroplast spacer for all three species were highly conserved, with 15 variable sites (2.1% of 854 bp) (Table 2). *Begonia formosana* shared with *B. aptera* 7 derived characters (at sites 43, 44, 251, 252, 280, 568, 743). Two autapomor-

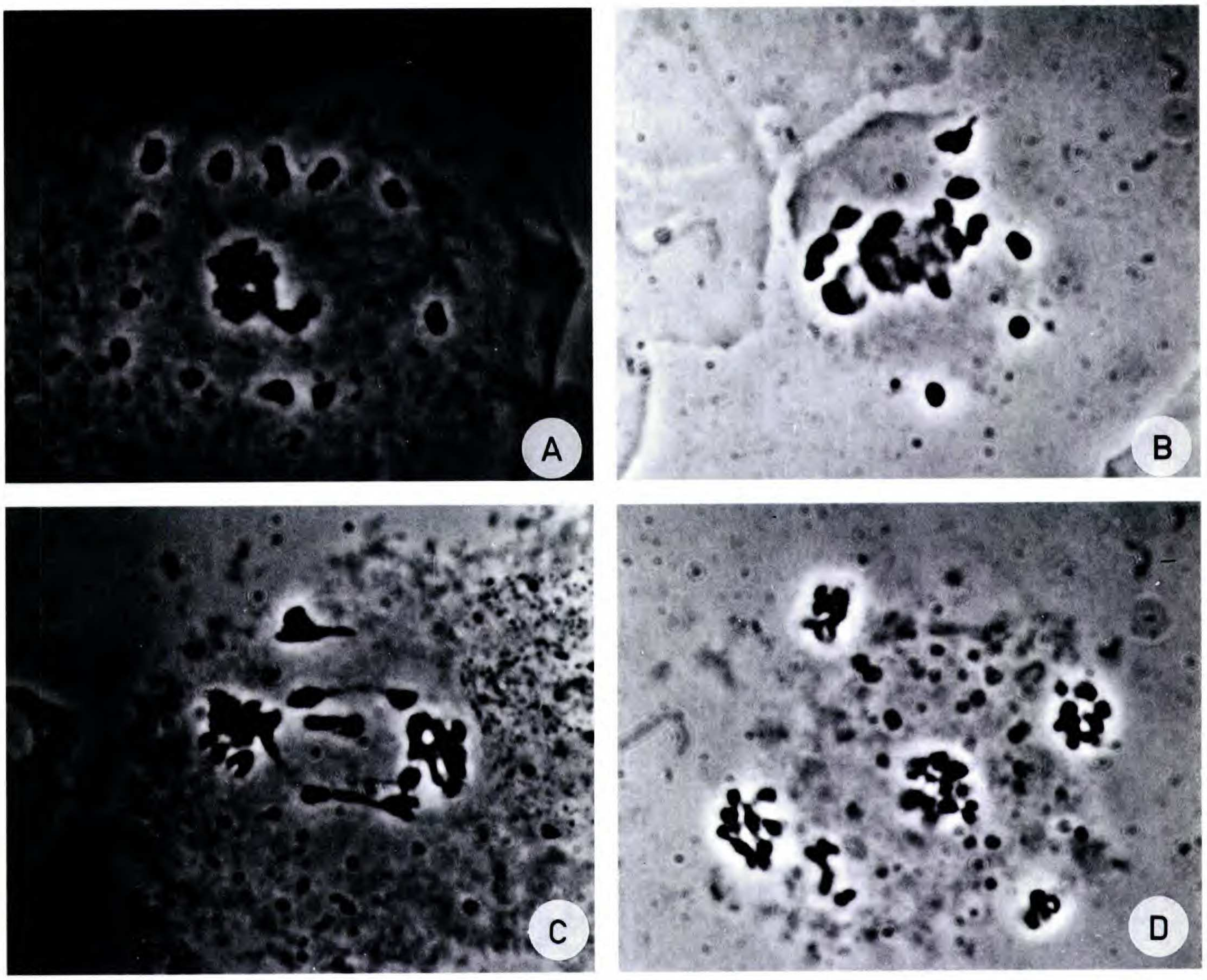


Figure 2. Meiotic chromosome spreads of *Begonia*.—A. Metaphase I, polar view. —B. Metaphase I, equatorial view. —C. Anaphase I. —D. Telophase II. Bar equals 10 μm (A, *Begonia* × *taipeiensis*, from *Peng 13899*; B–D, *B. formosana* × *B. aptera*, from *Leu 867* × *Peng 16153*).

Table 2. Variable sites of the nucleotide sequences of the *atp*B-*rbc*L spacer region of the chloroplast DNA of *Begonia* species. Dots indicate that the nucleotide sequences are identical to those for *Begonia aptera 16321*.

| Taxa/Sites | 43 | 44 | 191 | 251 | 252 | 270 | 275 | 286 | 298 | 371 | 376 | 568 | 743 | 762 | 794 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *B. aptera 16321* | C | G | T | T | A | A | A | A | G | T | T | C | T | T | G |
| *B. aptera 16276* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *B. aptera 16153* | . | . | . | . | . | . | G | . | . | . | . | . | . | . | C |
| *B. aptera 16292* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *B. taipeiensis 15106* | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *B. taipeiensis 13899* | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *B. taipeiensis 16320* | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *Bf*×*a*-2 | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *Bf*×*a*-1 | . | . | C | . | . | . | . | . | A | G | C | . | . | C | . |
| *B. formosana 13915* | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *B. formosana 16319* | . | . | . | . | . | . | . | . | A | G | C | . | . | . | . |
| *B. formosana 867* | . | . | C | . | . | . | . | . | A | G | C | . | . | C | . |
| *B. palmata 16081* | T | A | C | A | C | T | . | C | A | . | . | T | A | . | . |
| *B. palmata 16831* | T | A | C | . | C | T | . | C | A | . | . | T | A | . | . |

Table 3. Pairwise comparisons of the numbers of nucleotide subsitutions per site (K) in *Begonia* species (above diagonal). 1. *B. aptera 16321*; 2. *B. aptera 16276*; 3. *B. aptera 16153*; 4. *B. aptera 16292*; 5. *B. taipeiensis 15106*; 6. *B. taipeiensis 13899*; 7. *B. taipeiensis 16320*; 8. *Bf*×*a-2*; 9. *Bf*×*a-1*; 10. *B. formosana 13915*; 11. *B. formosana 16319*; 12. *B. formosana 867*; 13. *B. palmata 16081*; 14. *B. palmata 16831*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 0.0000 | 0.0011 | 0.0000 | 0.0017 | 0.0017 | 0.0017 | 0.0017 | 0.0028 | 0.0017 | 0.0017 | 0.0028 | 0.0056 | 0.0050 |
| 2 | | | 0.0011 | 0.0000 | 0.0017 | 0.0017 | 0.0017 | 0.0017 | 0.0028 | 0.0017 | 0.0017 | 0.0028 | 0.0056 | 0.0050 |
| 3 | | | | 0.0011 | 0.0028 | 0.0028 | 0.0028 | 0.0028 | 0.0039 | 0.0028 | 0.0028 | 0.0039 | 0.0067 | 0.0061 |
| 4 | | | | | 0.0017 | 0.0017 | 0.0017 | 0.0017 | 0.0028 | 0.0017 | 0.0017 | 0.0028 | 0.0056 | 0.0050 |
| 5 | | | | | | 0.0000 | 0.0000 | 0.0011 | 0.0000 | 0.0000 | 0.0011 | 0.0061 | 0.0056 | |
| 6 | | | | | | | 0.0000 | 0.0011 | 0.0000 | 0.0000 | 0.0011 | 0.0061 | 0.0056 | |
| 7 | | | | | | | | 0.0011 | 0.0000 | 0.0000 | 0.0011 | 0.0061 | 0.0056 | |
| 8 | | | | | | | | | 0.0011 | 0.0000 | 0.0000 | 0.0011 | 0.0061 | 0.0056 |
| 9 | | | | | | | | | | 0.0011 | 0.0011 | 0.0000 | 0.0061 | 0.0056 |
| 10 | | | | | | | | | | | 0.0000 | 0.0011 | 0.0061 | 0.0056 |
| 11 | | | | | | | | | | | | 0.0011 | 0.0061 | 0.0056 |
| 12 | | | | | | | | | | | | | 0.0061 | 0.0056 |
| 13 | | | | | | | | | | | | | | 0.0006 |

phies, at positions 371 and 376, in *B. formosana* distinguished it from *B. aptera.*

The sequences of *Leu 867* and *Peng 13915*, both *B. formosana*, differed at two sites. The genetic uniqueness of the two collections was transmitted to the $F_1$ offspring, i.e., B*f*×*a*-1 and B*f*×*a*-2, respectively, when they were used as maternal parent in experimental crosses. We sampled two collections of *Begonia* × *taipeiensis*, *Peng 13899* and *15106*, both associated with *B. formosana*, *Peng 13915*, from the same locality. Both *Begonia* × *taipeiensis* specimens were found to have identical sequences (Table 2) with the experimental hybrid (B*f*×*a*-2).

Parsimony analysis identified two equally parsimonious trees of 16 steps (Fig. 3), a CI of 0.938 ($P < 0.01$), and an RI of 0.958. A g1 statistic of −1.468 indicated a significant signal ($P < 0.01$). The Neighbor-Joining tree (Fig. 4), recovered by MEGA, based on the Kimura's two-parameter distance (Table 4), is consistent with the parsimony trees, but with higher resolution. Three major clades in both parsimony and NJ trees (Figs. 3, 4) obtained in these analyses were supported significantly by bootstrapping: the clade of *B. formosana*, *B.* × *taipeiensis*, and an experimental hybrid (B*f*×*a*-2), the clade of *B. formosana* (*Leu 867*) and an experimental hybrid (B*f*×*a*-1), and the clade of *B. aptera.* Within the first clade the monophyly of the subgroup composing the maternal parent (*B. formosana, Peng 16319*), the hybird offspring (B*f*×*a*-1), and another *B. formosana* (*Peng 13915*) was also significantly supported.

## Discussion

### Hybrid origin in *B.* × *taipeiensis*: evidence from morphology, cytology, and distribution

Based on morphological criteria, *B. formosana* and *B. aptera* were initially suggested as the putative parents of *B.* × *taipeiensis* (Peng & Sue, 2000). Cytological data showed that both the experimental hybrids between *B. formosana* ($n = 30$) and *B. aptera* ($n = 11$) and the naturally occurring *B.* × *taipeiensis* have the same chromosome number of $2n = 41$. *Begonia formosana* is the only species with $n = 30$ in Taiwan, which is the highest chromosome number among these *Begonia.* Chromosome numbers of $n = 11$, 15, 18, and 19 are known for other members of *Begonia* on the island (Y. K. Chen, 1988). A chromosome number of $n = 11$ is documented in only two species, *B. aptera* and *B. palmata* (Y. K. Chen, 1988; Peng & Chen, 1991). *Begonia palmata* was excluded as a candidate for a putative parent of *B.* × *taipeiensis* based on the geographic distribution of the species. Extensive fieldwork and examination of herbarium material revealed that the ranges of *B. aptera* and *B. formosana* largely overlap and that plants of *B.* × *taipeiensis* usually co-occur with these two species at ca. 60–200 m in elevation. On the other hand, *B. palmata*, mainly a montane species of higher altitudes (ca. 600–2100 m), is geographically and altitudinally disjunct. Based on data from experimental crosses, cytological observation, and geographical distribution, we conclude that *B.* × *taipeiensis* represents F1 progeny from natural hybridization between *B. formosana* and *B. aptera.*

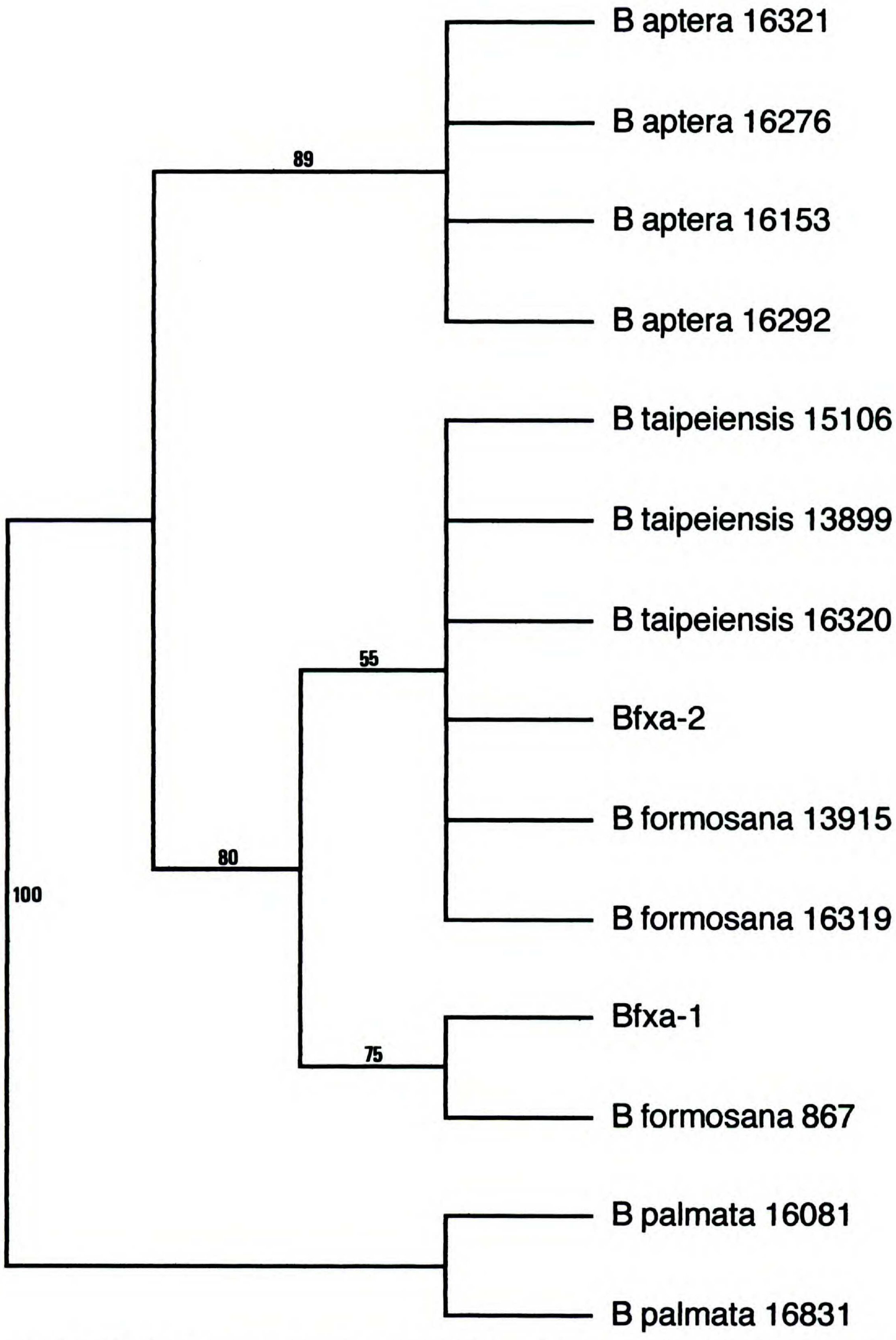


Figure 3. One of the parsimonious trees recovered by PAUP from nucleotide sequences of the *atp*B-*rbc*L spacer of chloroplast DNA rooted at *Begonia palmata*. Numbers at nodes are bootstrap values.

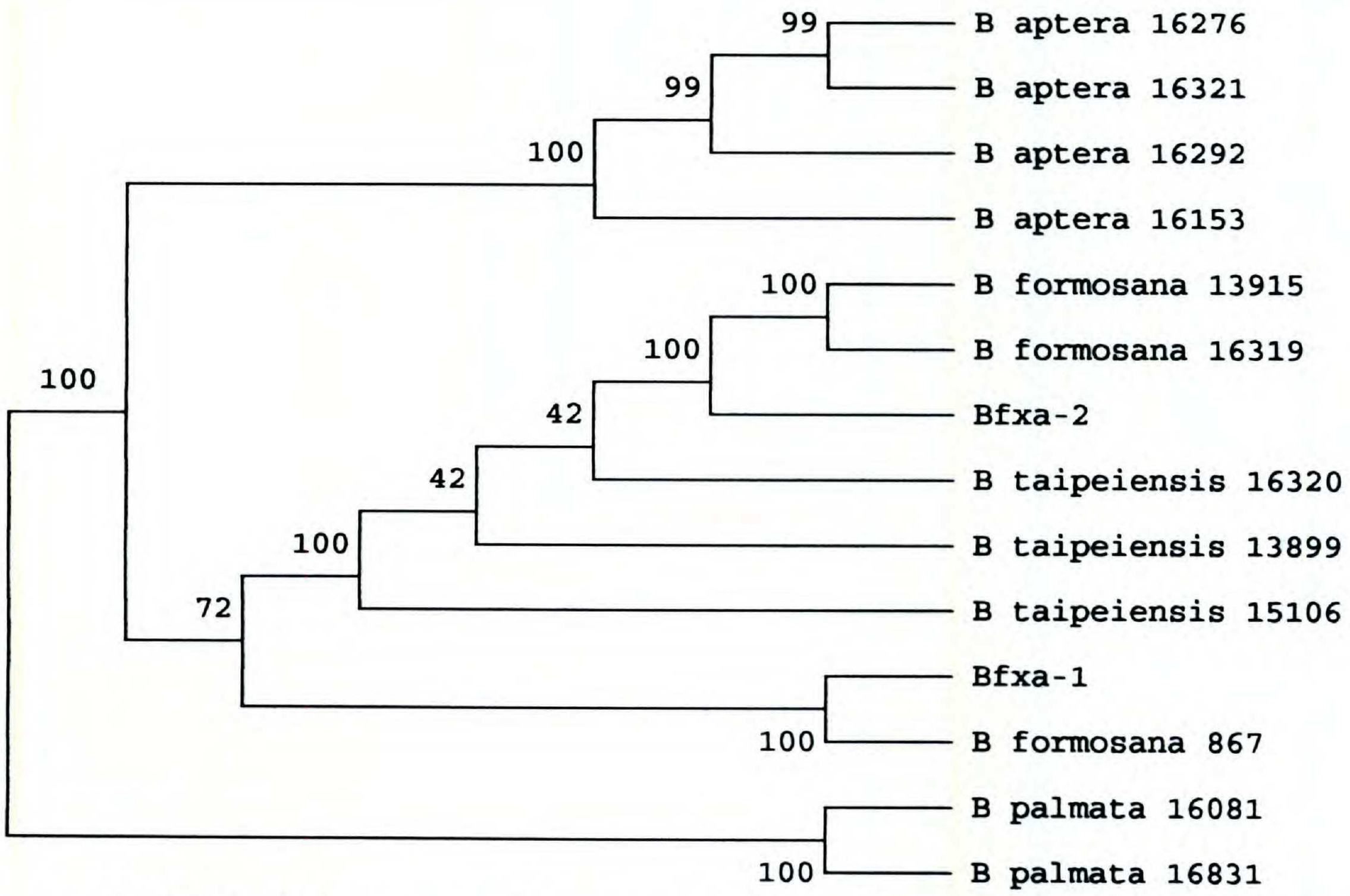


Figure 4. Neighbor-Joining tree recovered by MEGA from nucleotide sequences of *atp*B-*rbc*L spacer of chloroplast DNA rooted at *Begonia palmata*. Numbers at nodes are complete-and-partial (CP) bootstrap values.

### UNIDIRECTIONAL HYBRIDIZATION GIVING RISE TO *B.* × *TAIPEIENSIS*: EVIDENCE FROM MOLECULAR DATA AND EXPERIMENTAL HYBRIDIZATION

Identical sequences of the chloroplast *atp*B-*rbc*L spacer between *B.* × *taipeiensis* and *B. formosana* suggested that the former had a maternal origin from the latter. Unidirectional hybridization giving rise to the natural hybrid *B.* × *taipeiensis* is congruent with the results from reciprocal crosses in which viable $F_1$'s were obtained only when *B. formosana* was used as the pistillate parent. Experimental crosses using *B. aptera* as the maternal parent resulted in precocious fruit drop, possibly as a result of genetic disharmonies. A similar observation was made on Lousiana irises (Arnold & Bennett, 1993), in which unidirectional hybridization was documented using cpDNA haplotypes. Naturally occurring unidirectional hybridization suggests that hybrids of reversed parentages may have different survivorship, because organelle genomes may contribute genetic information that critically affects the survivorship of their progeny.

Compared to other organisms, such as in Hylocomiaceae (K = 0.012; Chiang, 1994) and Rubiaceae (K = 0.027; Manen & Natali, 1995), the chloroplast genetic variation between species of *Begonia* appears to be low. The distinctness of *B. formosana* supported by two derived sites (371 and 376) and that of *B. aptera* supported by a shared derived site (298) indicated, however, that this spacer is an appropriate marker at species level. The low level of interspecific variation in chloroplast DNA in Taiwanese *Begonia* may be ascribed to the abundant and recent natural hybridization and introgression events (Liu, 1999), which facilitated the morphological evolution and evolution of nuclear DNA via genetic recombination (based on our preliminary assessment of RAPD and nrDNA ITS sequence data). The possibility that the evolutionary rate of morphological changes may have surpassed that of the chloroplast DNA was similarly demonstrated in oaks with frequent natural hybridization (Whittemore & Schaal, 1991).

### FIT OR UNFIT, AND RECURRENT HYBRIDIZATION OF *B.* × *TAIPEIENSIS*

Natural hybrids may be considered to be impoverished genetically (cf. D. E. Soltis & P. S. Soltis, 1993; Arft & Ranker, 1998). Whether natural hybrids are more or less fit relative to their parents is a controversial issue (Arnold & Hodges, 1995). Experimental hybridization in this study revealed that there are very few if any pre- and post-zygotic reproductive barriers between these *Begonia*. We

Table 4. Kimura 2-parameter distance (above diagonal) and standard errors (below diagonal) of nucleotide sequences of *atp*B-*rbc*L noncoding spacer in *Begonia*. 1. *B. aptera 16321*; 2. *B. aptera 16276*; 3. *B. aptera 16153*; 4. *B. aptera 16292*; 5. *B. taipeiensis 15106*; 6. *B. taipeiensis 13899*; 7. *B. taipeiensis 16320*; 8. *Bf*×*a-2*; 9. *Bf*×*a-1*; 10. *B. formosana 13915*; 11. *B. formosana 16319*; 12. *B. formosana 867*; 13. *B. palmata 16081*; 14. *B. palmata 16831*.

| OTUs | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 0.0000 | 0.0024 | 0.0000 | 0.0035 | 0.0035 | 0.0035 | 0.0035 | 0.0059 | 0.0035 | 0.0035 | 0.0059 | 0.0118 | 0.0106 |
| 2 | 0.0000 | | 0.0024 | 0.0000 | 0.0035 | 0.0035 | 0.0035 | 0.0035 | 0.0059 | 0.0035 | 0.0035 | 0.0059 | 0.0118 | 0.0106 |
| 3 | 0.0017 | 0.0017 | | 0.0024 | 0.0059 | 0.0059 | 0.0059 | 0.0059 | 0.0083 | 0.0059 | 0.0059 | 0.0083 | 0.0142 | 0.0130 |
| 4 | 0.0000 | 0.0000 | 0.0017 | | 0.0035 | 0.0035 | 0.0035 | 0.0035 | 0.0059 | 0.0035 | 0.0035 | 0.0059 | 0.0118 | 0.0106 |
| 5 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | | 0.0000 | 0.0000 | 0.0000 | 0.0024 | 0.0000 | 0.0000 | 0.0024 | 0.0130 | 0.0118 |
| 6 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | 0.0000 | | 0.0000 | 0.0000 | 0.0024 | 0.0000 | 0.0000 | 0.0024 | 0.0130 | 0.0118 |
| 7 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | 0.0000 | 0.0000 | | 0.0000 | 0.0024 | 0.0000 | 0.0000 | 0.0024 | 0.0130 | 0.0118 |
| 8 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | 0.0000 | 0.0000 | 0.0000 | | 0.0024 | 0.0000 | 0.0000 | 0.0024 | 0.0130 | 0.0118 |
| 9 | 0.0026 | 0.0026 | 0.0031 | 0.0026 | 0.0017 | 0.0017 | 0.0017 | 0.0017 | | 0.0024 | 0.0024 | 0.0000 | 0.0130 | 0.0118 |
| 10 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0017 | | 0.0000 | 0.0024 | 0.0130 | 0.0118 |
| 11 | 0.0020 | 0.0020 | 0.0026 | 0.0020 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0017 | 0.0000 | | 0.0024 | 0.0130 | 0.0118 |
| 12 | 0.0026 | 0.0026 | 0.0031 | 0.0026 | 0.0017 | 0.0017 | 0.0017 | 0.0017 | 0.0000 | 0.0017 | 0.0017 | | 0.0130 | 0.0118 |
| 13 | 0.0037 | 0.0037 | 0.0041 | 0.0037 | 0.0039 | 0.0039 | 0.0039 | 0.0039 | 0.0039 | 0.0039 | 0.0039 | 0.0039 | | 0.0012 |
| 14 | 0.0036 | 0.0036 | 0.0039 | 0.0036 | 0.0037 | 0.0037 | 0.0037 | 0.0037 | 0.0037 | 0.0037 | 0.0037 | 0.0037 | 0.0012 | |

would expect natural hybrids when species of *Begonia*, such as *B. aptera* and *B. formosana*, co-occur and have overlapping flowering periods. *Begonia* taxa are also well known for their capability for vegetative propagation: they easily proliferate from fragments of stems, rhizomes, or leaves. Hybrid populations of *B.* × *taipeiensis*, however, are of sporadic occurrence (Fig. 1). Furthermore, such hybrids drop staminate flowers precociously and are completely seed-sterile. Their sterility, small population size, and rare occurrence suggest that they are less fit than the parental species, *B. aptera* and *B. formosana*. Many recent studies also indicate that natural hybrids are usually less competitive than their parental species, unless a novel niche can be explored (Arnold, 1993; Arnold & Hodges, 1995).

*Begonia* × *taipeiensis* was not known until very recently (Peng & Sue, 2000). It is seed-sterile, of limited distribution, and co-occurs with its parental species. Because it sheds staminate flowers precociously, we used it as an ovule donor to make experimental backcrosses with both of its parental species to test the possiblilty of introgression. Such attempts consistently failed to produce viable seeds. This, plus the sterility in *B.* × *taipeiensis*, led us to suggest that it can only persist through recurrent hybridization. Many recent molecular studies also suggest that recurrent hybridization events may occur over short spans of time (Ashton & Abbott, 1992; D. E. Soltis & P. S. Soltis, 1993; Arft & Ranker, 1998).

Although *B.* × *taipeiensis* is highly sterile, significant genetic variability could have been incorporated and accumulated into this hybrid from genetically distinct parental species (cf. Arft & Ranker, 1998). In nature, *B.* × *taipeiensis* with low frequency of fertility, which may not have been detected due to limited sampling, may occur. Similarly, the low level of viable seeds in backcrossings did not rule out the possibility of introgression in wild populations. Further study with wider and more intense samplings will be able to provide insight into the impact or potential of rarely occurring events on the evolution of Taiwanese *Begonia*.

In conclusion, when analyzed in concert, the data suggest that the formation of the natural hybrid *B.* × *taipeiensis* occurs via pollen transfer from *B. aptera* to the maternal species, *B. formosana*. Unidirectional hybridization suggests that differential survivorship exists between hybrids with reversed maternal origins. Even considering the ease with which experimental crosses are obtained, natural hybrids appear less fit than the parental species in this study, based on their sterility.

Literature Cited

Arft, A. M. & T. A. Ranker. 1998. Allopolyploid origin and population genetics of the rare orchid *Spiranthes diluvialis*. Amer. J. Bot. 85: 110–122.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. Ann. Rev. Ecol. Syst. 23: 237–261.

———. 1993. *Iris nelsonii* (Iridaceae): Origin and genetic composition of a homoploid hybrid species. Amer. J. Bot. 80: 577–583.

———. 1997. Natural Hybridization and Evolution. Oxford Univ. Press, New York.

——— & B. D. Bennett. 1993. Natural hybridization in Louisiana Irises: Genetic variation and ecological determinants. Pp. 115–139 *in* R. G. Harrison (editor), Hybrid Zones and the Evolutionary Process. Oxford Univ. Press, New York.

——— & S. A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. 10: 67–71.

———, B. D. Bennett & E. A. Zimmer. 1990. Natural hybridization between *Iris fulva* and *I. hexagona*: Pattern of ribosomal DNA variation. Evolution 44: 1512–1521.

Ashton, P. & R. J. Abbott. 1992. Multiple origins and genetic diversity in the newly arisen allopolyploid species, *Senecio cambrensis* Rosser (Compositae). Heredity 68: 25–32.

Baldwin, B. C. 1997. Adaptive radiation of the Hawaiian silversword alliance: Congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. Pp. 103–128 *in* T. J. Givnish & K. J. Sytsma (editors), Molecular Evolution and Adaptive Radiation. Cambridge Univ. Press, Cambridge.

Brochmann, C., Q. Y. Xiang, S. J. Brunsfeld, D. E. Soltis & P. A. Soltis. 1998. Molecular evidence for polyploid origins in *Saxifraga* (Saxifragaceae): The narrow Arctic endemic *S. svalbardensis* and its widespread allies. Amer. J. Bot. 85: 135–143.

Chen, C. H. 1993. Begoniaceae. Pp. 845–854 *in* Editorial Committee of the Flora of Taiwan, Flora of Taiwan, 2nd ed., Vol. 3. Editorial Committee of the Flora of Taiwan. Taipei.

Chen, Y. K. 1988. A Systematic Study of *Begonia* L. (Begoniaceae) of Taiwan. Master's Thesis, Graduate School of International Development of China, Section of Agriculture, Chinese Culture University, Taipei.

Chiang, T. Y. 1994. Phylogenetics and Evolution of the Hylocomiaceae (Mosses, Order Hypnales). Ph.D. Dissertation, Department of Biology, Washington University, St. Louis.

———, C. I Peng & B. A. Schaal. 1998. Universal primers for amplification and sequencing of *atp*B-*rbc*L noncoding spacer of chlroplast DNA. Bot. Bull. Acad. Sin. 39: 245–250.

Chien, A., D. B. Edgar & J. M. Trela. 1976. Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. J. Bacteriol. 127: 1550–1557.

Chiu, W. & B. B. Sears. 1985. Recombination between chloroplast DNAs does not occur in sexual crosses of *Oenothera*. Molec. Gen. Genet. 198: 525.

DeMarais, B. D., T. E. Dowling, M. E. Douglas, W. L. Minckley & P. C. Marsh. 1992. Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: Implications for evolution and conservation. Proc. Natl. Acad. Sci. U.S.A. 89: 2747–2751.

Doyle, J. J. & J. L. Doyle. 1989. A rapid DNA isolation

procedure for small quantities of fresh tissue. Phytochem. Bull. 19: 11–15.

———, J. L. Doyle, J. P. Grace & A. H. D. Brown. 1990. Multiple origins of polyploids in the *Glycine tabacina* complex inferred from chloroplast DNA polymorphism. Proc. Natl. Acad. Sci. U.S.A. 87: 714–717.

Farris, J. S. 1989. The retention index and the rescaled consistency index. Cladistics 5: 417–419.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Francisco-Ortega, J., D. J. Crawford, A. Santos-Guerra & R. K. Jansen. 1997. Origin and evolution of *Argyranthemum* (Asteraceae: Anthemideae) in Macaronesia. Pp. 407–431 *in* T. J. Givnish & K. J. Sytsma (editors), Molecular Evolution and Adaptive Radiation. Cambridge Univ. Press, Cambridge.

Garcia, D. K. & S. K. Davis. 1994. Evidence for a mosaic hybrid zone in the grass shrimp *Palaemonetes kadiakensis* (Palaemonidae) as revealed by multiple genetic markers. Evolution 48: 376–391.

Golenberg, E. M., M. T. Clegg, M. L. Durbin, J. Doebley & D. P. Ma. 1993. Evolution of a noncoding region of the chloroplast genome. Molec. Phylogen. Evol. 2: 52–64.

Harris, S. A. & R. Ingram. 1992. Molecular systematics of the genus *Senecio* L.: I. hybridization in a British polyploid complex. Heredity 69: 1–10.

Hedges, S. B. 1992. The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. Molec. Biol. Evol. 9: 366–369.

Higgins, D. G., A. J. Bleasby & R. Fuchs. 1992. CLUSTAL V: Improved software for multiple sequence alignment. CABIOS 8: 189–191.

Hillis, D. M. & J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42: 182–192.

——— & J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Hered. 83: 189–195.

Hodges, S. A., J. M. Burke & M. L. Arnold. 1996. Natural formation of *Iris* hybrid: Experimental evidence on the establishment of hybrid zone. Evolution 50: 1504–1509.

Huelsenbeck, J. P. 1991. Tree-length distribution skewness: An indicator of phylogenetic information. Syst. Zool. 40: 257–270.

Hughes, C. E. & S. A. Harris. 1994. The characterisation and identification of a naturally occurring hybrid in the genus *Leucaena* (Leguminosae: Mimosoideae). Pl. Syst. Evol. 192: 177–197.

Jackson, R. C. 1973. Chromosomal evolution in *Haplopappus gracilis*: A centric transposition race. Evolution 27: 243–256.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Molec. Evol. 16: 111–120.

Klassen, G. J., R. D. Mooi & A. Locke. 1991. Consistency indices and random data. Syst. Zool. 40: 446–457.

Kluge, A. G. & J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18: 1–32.

Kumar, P. S., K. Tamura & M. Nei. 1993. MEGA: Molecular Evolutionary Genetics Analysis, version 1.01. The Pennsylvania State University, Pennsylvania.

Li, W. H. 1997. Molecular Evolution. Sinauer Associates, Sunderland, Massachusetts.

——— & A. Zharkikh. 1995. Statistical tests of DNA phylogenies. Syst. Biol. 44: 49–63.

Liu, S. L. 1999. Multiple Origin of *Begonia formosana* Based on Nucleotide Variation of Chloroplast DNA *atp*B-*rbc*L Spacer and nrDNA ITS. Master's Thesis, National Cheng-Kung University, Tainan, Taiwan.

Manen, J. F. & A. Natali. 1995. Comparison of the evolution of ribulose-1, 5-biphosphate carboxylase (*rbc*L) and *atp*B-*rbc*L noncoding spacer sequences in a recent plant group, the tribe Rubieae (Rubiaceae). J. Molec. Evol. 41: 920–927.

Marchuk, D., M. Drumm, A. Saulino & F. S. Collins. 1991. Construction of T-vectors: A rapid and general system for direct cloning of unmodified PCR products. Nucl. Acids Res. 19: 1154.

Mummenhoff, K. & H. Hurka. 1995. Allopolyploid origin of *Arabidopsis suecica* (Fries) Norrlin: Evidence from chloroplast and nuclear genome markers. Bot. Acta 108: 449–456.

Murray, V. 1989. Improved double-stranded DNA sequencing using the linear polymerase chain reaction. Nucl. Acids Res. 17: 8889.

Ohsumi, C., A. Kojima, K. Hinata, T. Etoh & T. Hayashi. 1993. Interspecific hybrid between *Allium cepa* and *Allium sativum*. Theor. Appl. Genet. 85: 969–975.

Peng, C. I. 1988. The biosystematics of *Ludwigia* sect. *Microcarpium* (Onagraceae). Ann. Missouri Bot. Gard. 75: 970–1009.

———. 1990. *Ludwigia* × *taiwanensis* (Onagraceae), a new species from Taiwan, and its origin. Bot. Bull. Acad. Sin. 31: 343–350.

——— & Y. K. Chen. 1991. Hybridity and parentage of *Begonia buimontana* Yamamoto (Begoniaceae) from Taiwan. Ann. Missouri Bot. Gard. 78: 995–1001.

——— & C. Y. Sue. 2000. *Begonia* × *taipeiensis* (Begoniaceae), a new natural hybrid in Taiwan. Bot. Bull. Acad. Sin. 41: 151–158.

Pijl, L. van der. 1972. Principles of Dispersal in Higher Plants. Springer-Verlag, Berlin and Heidelberg.

Rieseberg, L. H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): Evidence from ribosomal genes. Amer. J. Bot. 78: 1218–1237.

——— & J. D. Morefield. 1995. Character expression, phylogenetic reconstruction, and the detection of reticulate evolution. Pp. 333–353 *in* P. C. Hoch & A. G. Stephenson (editors), Experimental and Molecular Approaches to Plant Biosystematics. Monogr. Syst. Bot. Missouri Bot. Gard. 53.

———, R. Carter & S. Zona. 1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* spp. (Asteraceae). Evolution 44: 1498–1511.

Sanger, F., S. Nicklen & A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74: 5463–5467.

Soltis, D. E. & P. S. Soltis. 1989. Allopolyploid speciation in *Tragopogon*: Insights from chloroplast DNA. Amer. J. Bot. 76: 1119–1124.

——— & ———. 1993. Molecular data and dynamic nature of polyploidy. Crit. Rev. Pl. Sci. 12: 243–273.

———, ——— & B. D. Ness. 1990. Maternal inheritance of the chloroplast genome in *Heuchera* and *Tolmiea* (Saxifragaceae). J. Hered. 81: 168–170.

Soltis, P. S. & D. E. Soltis. 1991. Multiple origins of the allotetraploid *Tragopogon mirus* (Compositae): rDNA evidence. Syst. Bot. 16: 407–413.

———, J. J. Doyle & D. E. Soltis. 1992. Molecular data and polyploid evolution in plants. Pp. 177–201 *in* P. S. Soltis, D. E. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants. Chapman and Hall, New York.

Stein, D. B. & D. S. Barrington. 1990. Recurring hybrid formation in a population of *Polystichum potteri*: Evidence from chloroplast DNA comparisons. Ann. Missouri Bot. Gard. 77: 334–339.

Swofford, D. L. 1991. When are phylogeny estimates from molecular and morphological data incongruent? Pp. 295–333 *in* M. M. Miyamoto & J. Cracraft (editors), Phylogenetic Analysis of DNA Sequences. Oxford Univ. Press, New York, Oxford.

———. 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.1. Computer program distributed by the Illinois Natural History Survey. Champaign, Illinois.

Vogelstein, B. & D. Gillespie. 1979. Preparation and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U.S.A. 76: 615–619.

Wang, X. R. & A. E. Szmidt. 1994. Hybridization and chloroplast DNA variation in a *Pinus* species complex from Asia. Evolution 48: 1020–1031.

Wendel, J. F., J. M. Stewart & J. H. Rettig. 1991. Molecular evidence for homoploid reticulate evolution among Australian species of *Gossypium*. Evolution 45: 694–711.

Whittemore, A. T. & B. A. Schaal. 1991. Interspecific gene flow in sympatric oaks. Proc. Natl. Acad. Sci. U.S.A. 88: 2540–2544.

Wu, C. I. & W. H. Li. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proc. Natl. Acad. Sci. U.S.A. 82: 1741–1745.